Northern blotting

Reagents: Agarose GTG: Cat # 50070, FMC BioProducts (Rockland, Maine)
10xMOPS:
37% formaldehyde
DEPC water
Ethidium bromide
Deionized formamide

Procedure:

1. Gel
   horizontal gel 14 slots, wide comb, 3.5ug per lane

Agarose gel	agarose GTG	1.2g
	H2O	87ml in Erlenmeyer
	Microwave until dissolved (~2min)	
	10xMOPS	10ml
	cool to touch	
	37% formaldehyde	5.1ml

   Mix above solution gently to avoid forming air bubbles. Pour all into gel box, wait until it cools to form a 0.5cm gel

2. Samples

a. Making buffer: 500ul

Sample buffer

a. formamide (deionized)

10x MOPS	150ul
DEPC water	150ul
40% formaldehyde	200ul
b. "northern dye"	200ul

Mix A and B, store at -20^0C

Diluted ethidium bromide

ethidium bromide	5ul
DEPC water	1ml

b. Loading samples

Label the sample tubes. Add 12ul sample buffer and 3ul diluted ethidium bromide (5 ul of 10 mg/ml in 1000 ul water) to all the tubes. Add sample and DEPC water according to below table. Heat the sample tubes at 65^0C for 5 minutes. Load all the sample (~25ul) into sample well (see map show bellow). Run the gel at 50Volts for 4-5 hours.

3. Blotting

    a. Rinse the gel twice with 10xSSC for 15 min. to remove formaldehyde, which increases transfer efficiency of the gel).
    b. Put blotting pad in a plastic container. Wet top 2-3 blotting pads with 10xSSC.
    c. Cut Zeta-Probe GT membrane to gel measurements (11x14 inches), wet with 10xSSC.
    d. Place the gel "upside-down" (RNA sink on the bottom of the gel) on wet pads, lay membrane on it, remove air bubbles between membrane and gel, put 2 wet pads above and cover with dry pads.
    e. Transfer for overnight.
    f. Observe the transfer result under UV. The bands are easily observed on the reverse side of RNA on the membrane. For a good transfer, no bands will be left in the gel.
    g. Link the RNA to membrane (stratalink) in UV-oven (autolink+start).
    h. Put the membrane into a pouch, store it at 4^0C.

4. Prehybridization

    a. Hybridization solution Hybrisol (ONCOR, Gaithersburg, MD)
    b. Preheat hybrisol at 55^0C (dissolve all ingredients) until it is clear.
    c. Put membrane into hybridization tube and add 15ml hybrisol.
    d. Prehybridization at 41^0C in oven with rotation for at least 3 hours.

5. Radiolabel probe

DNA	25ng (1ul)
Water	22ul
Random Oligonucleotide primers	10ul
Boil for 5 minutes at 95-100^0C, spin-down the liquid	
5x Primer buffer	10ul
□CTP^{32}	5ul
T7 DNA polymerase	1ul
Incubate for 5 minutes at 37^0C	
Blue sample buffer (optional) to stop the reaction (any labeling kit will do)	10ul

6. Purify the probe

    STE Select-D, G-50 spin column

    a. Invert column several time to fully resuspend gel, Remove top closure first, and then bottom one

b. Let the buffer drain, put it in a collection tube, and spin at 1000rpm for 1.5 min.
c. Change the collection tube, and discard the old one.
d. Put all the sample (~ 60ul) onto the center of gel bed.
e. Spin at 1000rpm for 1.5 min.
f. Check probe with Geigercounter: put 1ul of 4 ml of scintillation solution and count cpm. Use about 1 X 10 7/blot

7. Hybridization
    a. Put radioactive probe into a 0.5ml screw cap tube, boil the probe (1-2 x 10^6 cpm/ml Hybrosol) for 3 minutes in a heating block, put it into the hybridization tube with membrane, and put that into the hybridization oven.
    b. Incubate/rotate at 42^0C for overnight.
    c. Wash the membrane 2x for 15 minutes in 2x SSC/0.1% SDS at room temperature. This can be done in the hybridization oven.
    d. Wash the membrane 2x for 20 minutes in 0.1x SSC/0.1% SDS at 68^0C. This is also done in the hybridization oven

8. Autoradiography//other imaging analysis
    a. Let membrane dry briefly, wrap it, and put it in an X-ray cassette with and intensifying screen.
    b. Incubate at -70^0C (time depends on how "hot" the signal is by gieger). Develop the X-ray film in the developer on the 1st floor.
    c. Alternatively, place the membrane (covered with plastic wrap) in molecular dynamics imaging system for 1 h to 1 day.